THE EFFECT OF A SURFACTANT AND OF PARTICLE SIZE ON GRISEOFULVIN PLASMA LEVELS*

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The use of griseofulvin as a systemic antidermatophyte in humans is well established; however, only limited data are available relating to the efficiency and mode of its absorption. In an investigation of the absorption of griseofulvin in rats after oral administration, it was observed by Bedford *et al.* (1) that maximum blood levels were attained within four hours. They also found that tissue concentrations paralleled blood levels and that the absorption appeared to be self-limiting. After 24 hours, they were able to recover 16% of the administered dose in the feces as intact drug. When the feces and the entire alimentary tract were extracted, Davis (2) recovered 91% and 55% of the oral dose in rats after 4 and 24 hours, respectively.

Crounse (3) reported that blood levels of griseofulvin were variable in human subjects and could be enhanced when the drug was administered in conjunction with high fat meals. He concluded that inadequate absorption may be one of the mechanisms of clinical failure with ordinary therapeutic doses of griseofulvin. Other investigators (4, 5, 6) have also observed irregular blood levels and excretion rates after oral administration to humans. Riegelman (4) has been able to demonstrate greatly enhanced absorption by using a supersaturated solution of the drug in polyethylene glycol 400.

Surfactant Effect. It has been reported in the literature that surfactants promote the absorption of iron (7), spironolactone (8) and fat soluble substances (9) from the gastrointestinal tract. Kraml *et al.* (10) found that there was no increase of serum blood levels in rats when Perminal BXN (butylated sodium naphthalene sulfonate) was added to an aqueous suspension of griseofulvin. However, Duncan *et al.* (11) observed that the same surface active agent in aqueous suspension gave rise to higher blood levels. They found that the method of incorporating the surfactant into the preparation had a pronounced effect on the attained blood levels. The addition of Perminal BXN to tablets had no significant effect upon human blood levels.

Particle Size Effect. It has been shown by many investigators that particle size has a pronounced effect upon the biological activity of certain therapeutic agents. Douglas *et al.* (12) have demonstrated that in nematode infections of sheep, the particle size of phenothiazine is an important factor in determining its anthelminthic activity. He found that phenothiazine, having a particle diameter of 140 microns or larger, was ineffective. However, drug with a mean particle diameter between 40 and 50 microns was 70% effective and drug with a mean particle diameter between 1 and 2 microns was 95% effective. Investigations have shown that sulfadimethoxine (13), sulfadiazine (14), phenolphthalein (15) and sulfur (16) are more rapidly absorbed when administered in a microcrystalline dosage form.

Griseofulvin exhibits the low water solubility of approximately 1 mgm per 100 ml. This physical property may have a pronounced effect upon the availability of the drug for absorption and may be responsible for the self-limiting factor reported by Bedford (1). Since Riegelman found in humans that absorption could be enhanced when the drug was given in solution, we have investigated methods to enhance the drug's solubility in water and to modify the physical properties to promote availability for absorption. The two principal approaches pursued were reduction of particle size and solubilization by surfactants. We have attempted to study griseofulvin blood levels in rats, guinea pigs and human volunteers following the oral administration of the drug in solution, in different particle sizes and with the co-administration of a surfactant.†

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METHODS

Solubility.—The aqueous solubility of griseofulvin with and without sodium lauryl sulfate (SLS) were determined in a water bath at 30 ± 0.1°. Our findings are shown in Table I.

Biological Tests.—Male rats were given identical oral doses of griseofulvin as a solution in polyethylene glycol 400 or as an aqueous suspension. Blood samples were obtained by direct cardiac puncture from anesthetized animals. Each specimen for analysis represented the pooled blood from three rats. Plasma levels were determined according to the spectrophotofluorometric method of Bedford *et al.* (17). The results obtained are reported in Table II.

TABLE I

Solubilization of griseofulvin by SLS in water at 30°

% SLS	Griseofulvin (mg %)
0	1
0.25	17
0.5	41
1.0	91
2.0	171

TABLE II

Comparison of griseofulvin plasma levels following the oral administration of a solution and a suspension to rats

Dosage Form Administered	Dose (mg/Kg)	Plasma Levels in mcg/ml			
		2 Hrs	4 Hrs	6 Hrs	24 Hrs
Polyethylene glycol 400 Solution, 0.5%	50	2.4	2.2	2.3	0
Aqueous Suspension 0.5%	50	1.3	0.7	0.7	0

TABLE III

*Comparison of griseofulvin microbiological activity in blood following the oral administration of different particle sizes of drug with and without SLS to guinea pigs**

Preparation Administered	Dose (mg/Kg)	Average Blood Level (mcg/ml of activity)
Griseofulvin SSA 0.41 M^2/Gm	60	2.22
Griseofulvin SSA 0.41 M^2/Gm + SLS	60 + 12	3.26
Griseofulvin SSA 1.0 M^2/Gm	60	4.28
Griseofulvin SSA 1.0 M^2/Gm + SLS	60 + 12	3.87

* These biological studies were conducted by Dr. E. R. L. Gaughran.

Blood levels were determined in groups of three male guinea pigs following single oral doses of griseofulvin suspensions of two different particle sizes, with and without SLS. Six hours post administration, 2 ml blood samples were withdrawn and immediately assayed against *Trichophyton mentagrophytes* colonies of a reproducible size. Standard curves were simultaneously obtained by adding known amounts of griseofulvin to 2 ml samples of control blood. After five days incubation, cultures were compared. The results are reported as griseofulvin activity in Table III.

Human Protocol.—The methods of administering the drug and collecting samples were as follows.

In the studies with single oral doses, 12 healthy fasting male and female subjects were given four 250 mg tablets of one kind of griseofulvin (regular or microcrystalline*, with or without sodium lauryl sulfate) at 8 a.m. Blood samples were drawn into heparinized syringes at 0, 1, 4, 8, 24 and 48 hours after dosing. Each subject received all four kinds of griseofulvin at the same dose, at weekly intervals, and the order of administration was randomized by a Latin Square design.

When multiple doses were administered, groups of 6 or 12 healthy male volunteers were given a 125 mg or 250 mg tablet of one form of griseofulvin at 8 a.m., noon, 4 p.m. and 8 p.m. each day for a week (or for 29 days in the last study). Blood samples were obtained at 8 a.m. on the first day and at 4 p.m. on days 1–7. In the week-long studies, half of the subjects in the first week were always given one form of griseofulvin, the other half another form of the drug. An interval of a week without drug was permitted to elapse. The subjects were then crossed over to the second form of the drug in the third week. In the long-term crossover study, two weeks without drug intervened between the 29-day periods on drug. Blood samples in this study were obtained at 8 a.m. on the first day and at 4 p.m. on days 1, 2, 3, 4, 5, 8, 15, 22 and 29.

In this and all other studies, the plasma was removed after centrifugation and frozen. Duplicate assays of all samples on one subject were run on the same day.

Assay.—Plasma levels of griseofulvin were determined by the spectrophotofluorometric method of Bedford *et al.* (17). An Aminco-Bowman spectrophotofluorometer coupled to an X-Y Electro

* Regular griseofulvin as Grifulvin® McNeil and microcystalline griseofulvin as Grifulvin V® McNeil.

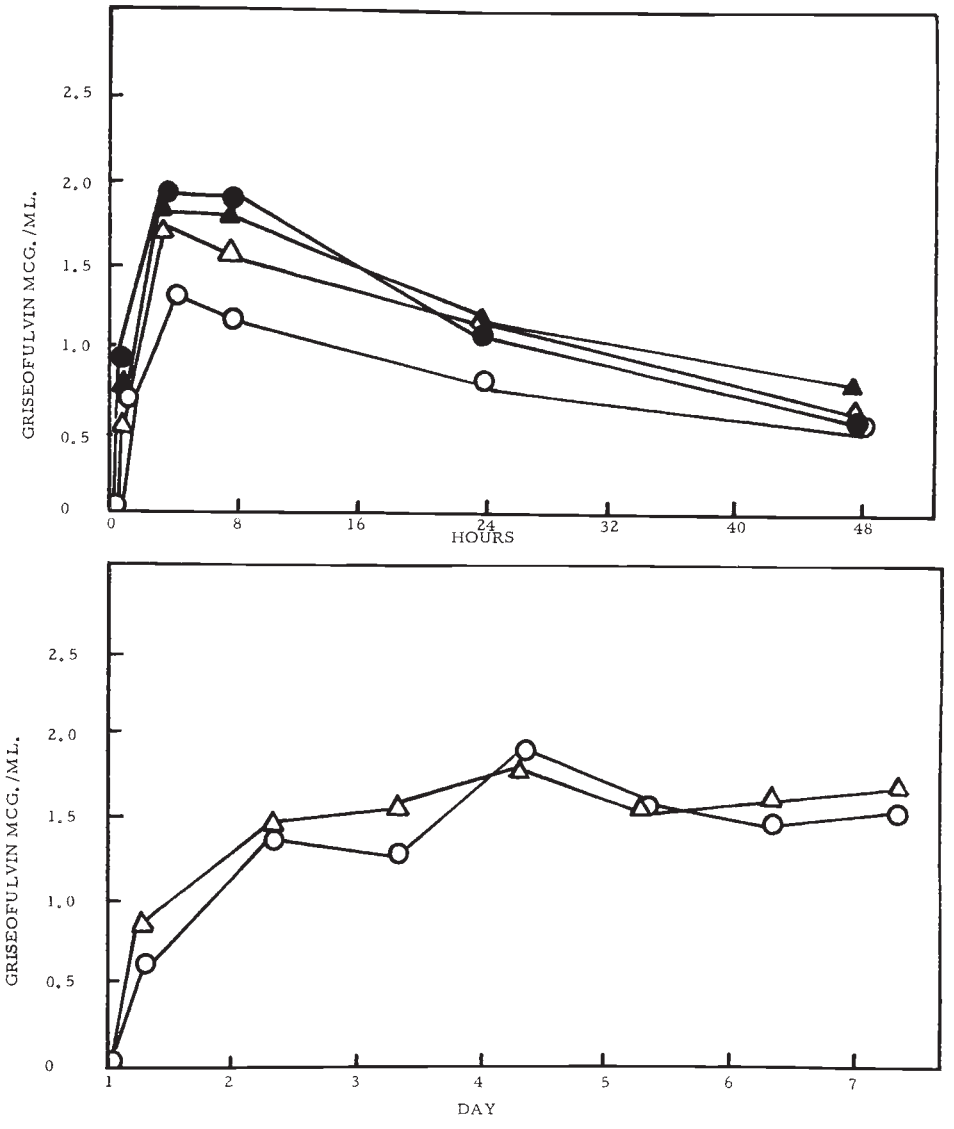

FIG. 1. A comparison of the effect of particle size and sodium lauryl sulfate (S.L.S.) on griseofulvin plasma levels after the administration of a single oral dose of one gram. ○ S.S.A. 0.41 M^2/Gm. △ S.S.A. 0.41 M^2/Gm. plus S.L.S. (200 mg.), average for twelve subjects. ● S.S.A. 1.0 M^2/Gm. ▲ S.S.A. 1.0 M^2/Gm. plus S.L.S. (200 mg.) average for twelve subjects.

FIG. 2. A comparison of the effect of sodium lauryl sulfate (S.L.S.) on griseofulvin plasma levels during the administration of repeated doses. ○ S.S.A. 0.41 M^2/Gm., 250 mg. tablets four times a day, average for five subjects. △ S.S.A. 0.41 M^2/Gm., 250 mg. plus S.L.S. 50 mg. tablets four times a day, average for five subjects.

Instruments recorder was used. It has been reported by Child *et al.* (18) and Marvel (Crounse, 3) that certain drugs in common usage, such as aspirin, interfere with the fluorometric analysis of griseofulvin. To ensure that all hydroalcoholic plasma extracts were contaminant-free, at least one sample for each plasma specimen was scanned between the analyzing wavelengths of 200 and 800 millimicrons. (For comparison with the English workers (1, 2, 18, 19 and 20), it should be noted that plasma levels by this assay are 40% higher than whole blood levels.)

Particle Size.—Griseofulvin mean particle diameters were determined using a Fisher sub-sieve sizer and specific surface areas were calculated therefrom.

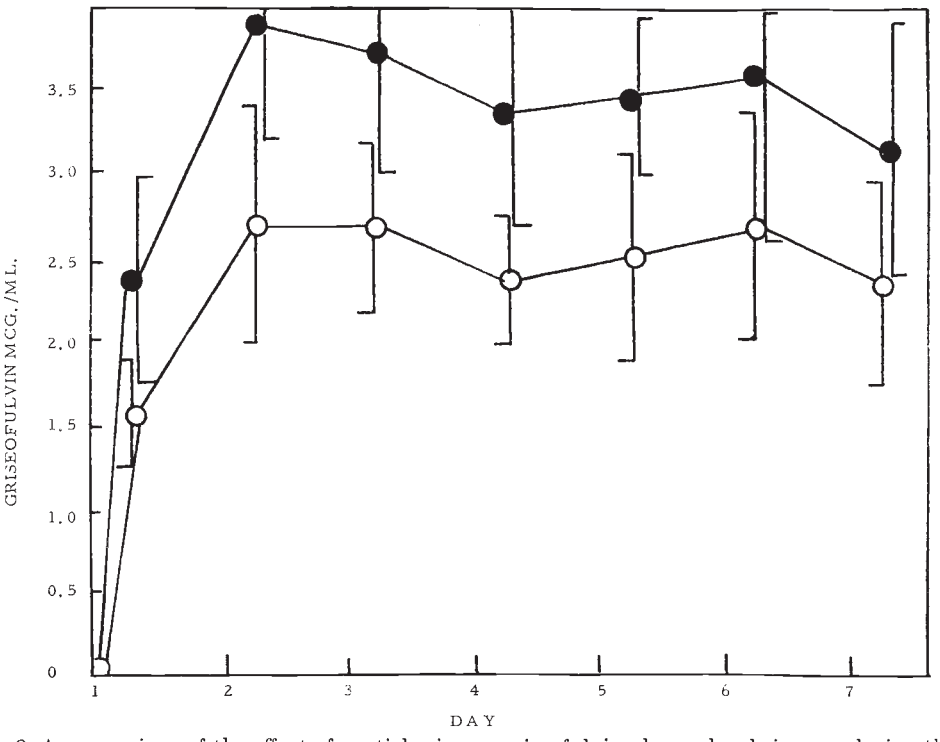

FIG. 3. A comparison of the effect of particle size on griseofulvin plasma levels in man during the administration of tablets. ○ S.S.A. 0.41 M^2/Gm., 250 mg. four times a day, average for eleven subjects. ● S.S.A. 1.46 M^2/Gm., 250 mg. four times a day average for eleven subjects.

RESULTS AND DISCUSSION

Sodium lauryl sulfate was chosen for this study because of its safety and solubilizing properties. The effect of SLS is to markedly increase the aqueous solubility of griseofulvin, as shown in Table I.

As shown in Table II, when the identical dose in the same volume of vehicle was administered orally to rats as a solution in polyethylene glycol 400 or as an aqueous suspension, higher plasma levels were obtained with the solution. The data obtained in our laboratories is in agreement with the work of Riegelman (4), and gives further credence to the fact that incomplete absorption occurs in rats after oral administration of the drug.

Table III shows the enhancing effect of reduced particle size and of SLS on griseofulvin microbiologic activity following oral administration to guinea pigs.

A comparison of the effect of SLS and of varying particle sizes in a griseofulvin tablet on plasma levels in humans was made and is reported in Fig. 1. The results indicate that either SLS or reduction of particle size will improve the plasma levels obtained with S.S.A. 0.41 M^2/Gm griseofulvin.

Since SLS increased griseofulvin levels in single oral doses in both guinea pigs and humans, a comparison was made using multiple daily doses over a prolonged period of time as shown in Fig. 2. Contrary to expectations, no enhancement occurred. Apparently, multiple dosing improved the efficiency of absorption as much as the surfactant could. Atkinson *et al.* (19) in studying dosage regimens have found that divided daily dosage results in higher blood levels than single daily doses. Consequently, one must design experiments so that the information obtained will be valid when applied to clinical programs. Caution must be used in interpreting data from single dose experiments.

Other investigators, using single oral doses, have reported on the effect of particle size on griseofulvin blood levels (11, 20, 21). As mentioned previously, using griseofulvin of large particle size, Atkinson *et al.* (19) obtained higher

TABLE IV

Plasma griseofulvin levels in mcg/ml in man on repeated oral doses of 250 mg tablets four times a day using S.S.A. 0.41 M²/Gm material

Day	Subject											Avg. ± S.D.
	1	2	3	4	5	6	7	8	9	10	11	
1	0.62	1.50	1.42	0.57	1.62	0.73	0.83	1.16	1.22	0.97	1.41	1.12 ± 0.41
	0.61	1.51	1.27	0.57	2.07	0.75	0.70	1.42	—	1.27	1.33	
2	—	2.05	1.68	1.15	3.20	1.42	1.43	2.34	2.60	2.23	3.35	2.14 ± 0.72
	—	2.21	1.62	1.02	2.82	—	1.47	2.82	2.48	1.65	3.20	
3	2.37	2.02	2.16	2.48	3.35	3.12	1.40	1.70	2.52	2.97	3.05	2.35 ± 0.58
	2.37	1.93	1.77	1.92	2.60	3.05	1.18	1.76	2.55	—	3.02	
4	2.94	1.80	2.60	1.67	3.08	2.97	0.86	1.70	2.45	3.54	2.90	2.29 ± 0.76
	2.94	1.63	2.21	1.55	3.08	—	0.86	1.57	2.52	2.45	2.86	
5	1.70	1.62	2.51	—	3.27	3.20	0.80	1.61	2.52	—	3.42	2.23 ± 0.84
	1.45	1.56	—	—	2.67	—	—	1.22	2.71	—	3.27	
8	1.47	1.73	2.02	1.30	3.08	1.76	1.17	1.20	2.41	—	2.97	1.90 ± 0.67
	1.45	1.73	1.95	1.24	2.93	—	1.05	1.18	2.45	—	2.93	
15	1.82	1.66	1.92	1.79	2.52	2.71	1.66	2.16	2.52	2.16	3.11	2.21 ± 0.47
	1.78	1.68	2.06	—	2.90	2.49	1.76	2.07	—	2.18	3.20	
22	1.46	1.86	1.46	1.41	2.31	2.13	1.27	2.15	2.90	2.48	2.56	2.02 ± 0.49
	1.45	1.72	1.58	1.91	2.32	2.07	1.31	2.21	2.82	2.32	2.71	
29	1.42	1.82	1.91	2.01	2.26	2.93	1.53	1.13	2.71	1.52	1.51	1.89 ± 0.53
	1.42	1.82	1.92	2.01	2.31	2.75	—	1.17	2.71	1.32	1.49	

TABLE V

Plasma griseofulvin levels in mcg/ml in man on repeated oral doses of 125 mg tablets four times a day using S.S.A. 1.45 M²/Gm material

Day	Subject											Avg. ± S.D.
	1	2	3	4	5	6	7	8	9	10	11	
1	1.23	1.33	0.70	0.95	1.10	1.00	0.76	1.47	0.53	0.61	0.96	1.01 ± 0.17
	1.21	1.40	0.76	0.83	0.97	1.12	0.85	1.70	—	0.70	0.97	
2	—	2.60	1.90	2.37	1.87	2.06	1.61	1.20	1.80	2.00	1.87	1.84 ± 0.39
	—	2.37	1.64	2.32	1.85	—	1.42	1.11	1.22	1.51	2.16	
3	1.45	3.05	1.56	1.08	2.26	3.07	2.23	1.83	1.56	1.75	2.00	2.00 ± 0.56
	1.50	2.41	1.51	1.11	2.15	3.05	1.77	2.31	2.06	—	2.25	
4	1.64	2.41	1.71	1.27	1.65	3.01	1.57	1.87	1.87	1.87	1.93	1.85 ± 0.42
	1.64	2.71	1.85	1.25	1.82	—	1.50	1.93	1.82	1.71	1.90	
5	1.49	2.60	1.68	—	2.05	3.80	1.64	2.00	1.83	—	2.45	2.06 ± 0.59
	1.49	2.30	—	—	2.00	—	—	1.58	1.65	—	2.37	
8	1.76	2.09	1.68	1.32	1.65	2.47	1.27	1.46	1.47	—	1.47	1.62 ± 0.28
	1.47	2.07	1.62	1.28	1.60	—	1.57	1.66	1.37	—	1.45	
15	1.93	1.42	2.05	—	1.68	2.41	1.02	1.72	1.59	1.66	2.05	1.77 ± 0.37
	2.22	1.45	1.66	1.54	1.61	2.67	1.48	1.57	—	1.71	1.98	
22	1.28	1.50	2.67	0.52	1.90	2.22	1.62	1.42	1.87	1.47	2.10	1.71 ± 0.58
	1.32	1.60	2.90	0.45	1.77	2.67	1.56	1.51	1.80	1.43	2.00	
29	1.52	2.22	2.10	1.74	1.45	1.97	1.40	1.40	1.90	1.02	2.01	1.70 ± 0.37
	1.42	2.11	2.37	1.64	1.45	2.05	—	1.35	1.77	0.96	1.87	

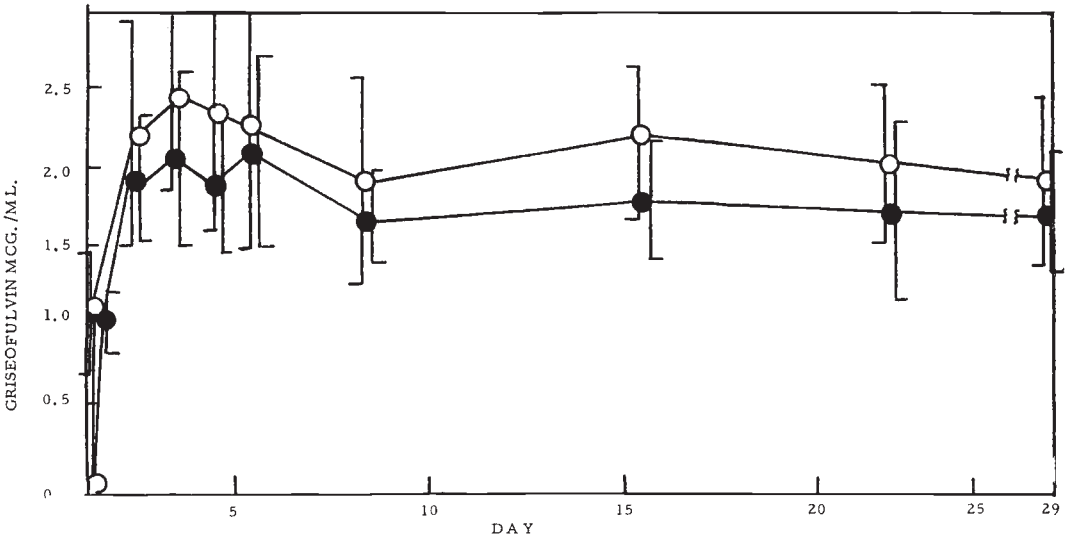

FIG. 4. A comparison of the effect of half-doses of microcystalline with full doses of standard griseofulvin on griseofulvin plasma levels in man during the administration of tablets. ○ S.S.A. 0.41 M^2/Gm., 250 mg. four times a day, average for eleven subjects. ● S.S.A. 1.45 M^2/Gm., 125 mg., four times a day, average for eleven patients.

blood levels with divided daily doses than with single daily doses. Having failed to find plasma level enhancement with surfactants when administered in a prolonged, divided dose schedule, we felt it imperative to study microcrystalline griseofulvin under multiple dose conditions for a prolonged period of time.

The effect of particle size on griseofulvin plasma levels was studied in humans as described in the protocol. The results are recorded in Fig. 3 and show that the microcrystalline material gives levels that are between 35 and 50% higher than the regular material when given four times a day as tablets for a week.

Half-doses of microcrystalline griseofulvin were compared with full doses of regular griseofulvin in a similar manner for one month. The results are recorded in Tables IV and V. The averages show no significant differences except on day four, when analyzed by Student's "t" test. Fig. 4 represents these results graphically.

We may conclude that significantly higher plasma levels are maintained using griseofulvin of greater surface area under clinical conditions. Plasma levels approximately equal to a full dose of standard griseofulvin can be maintained for a prolonged period of time using half-doses of microcrystalline griseofulvin.

SUMMARY AND CONCLUSIONS

The administration to guinea pigs and to human subjects of a single oral dose of regular griseofulvin with sodium lauryl sulfate (SLS) gave higher griseofulvin plasma levels than the drug lone. However, in man, when the same daily dose was divided and given each day for a week, the SLS had no enhancing effect on the attained plasma levels.

An increase in griseofulvin plasma levels in guinea pigs and human subjects was found when the particle size of the griseofulvin was reduced. Significantly higher levels were maintained for a week when one gram daily divided doses of griseofulvin S.S.A. 1.46 M^2/Gm were compared with griseofulvin S.S.A. 0.41 M^2/Gm. No significant difference could be found, except on day four, when half-doses of the microcrystalline griseofulvin were compared similarly with full doses of regular griseofulvin for a month.

Caution must be used in interpreting data from single dose studies. Experiments need to be designed so that the information obtained will be valid when applied to clinical use.

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